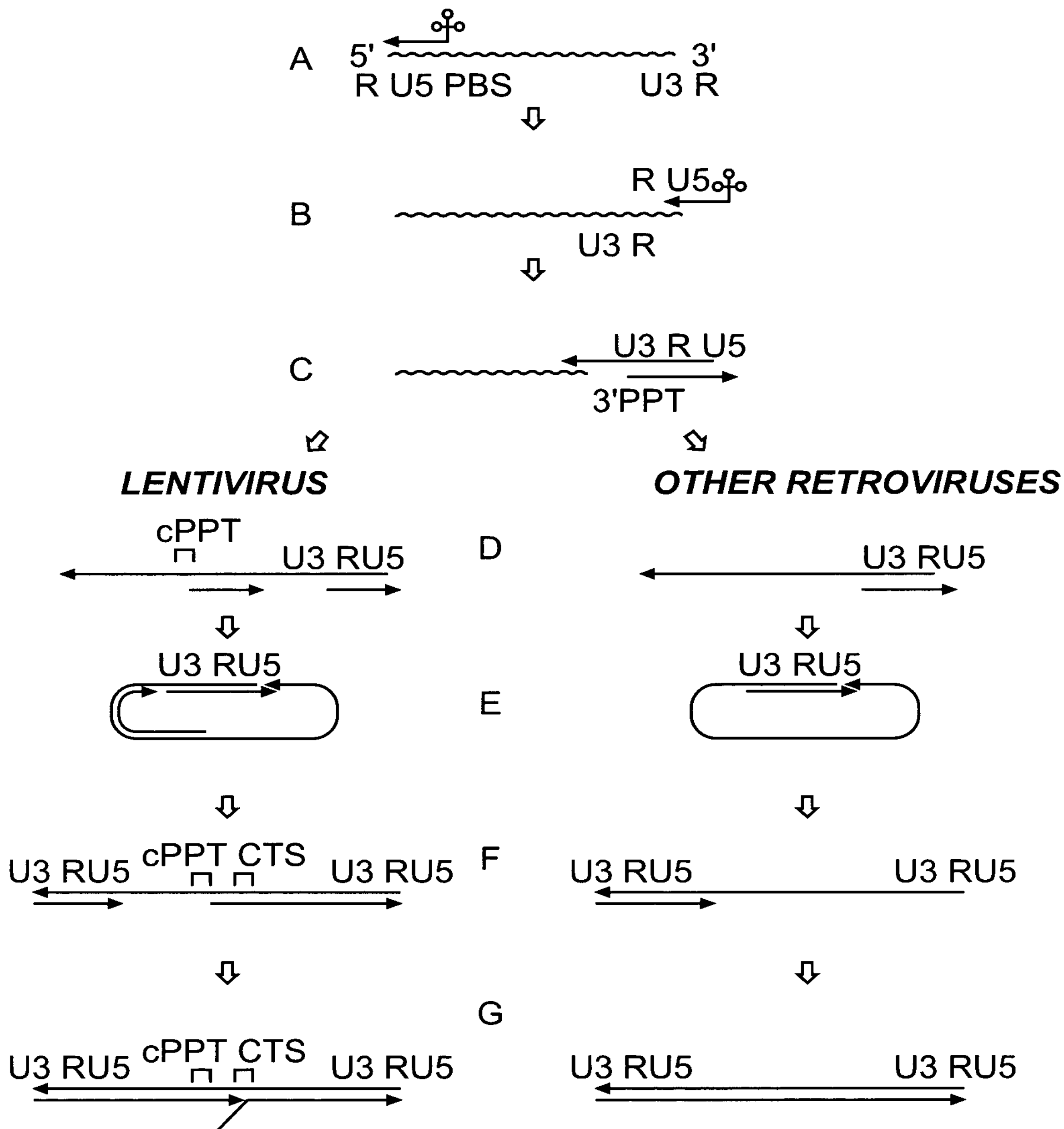
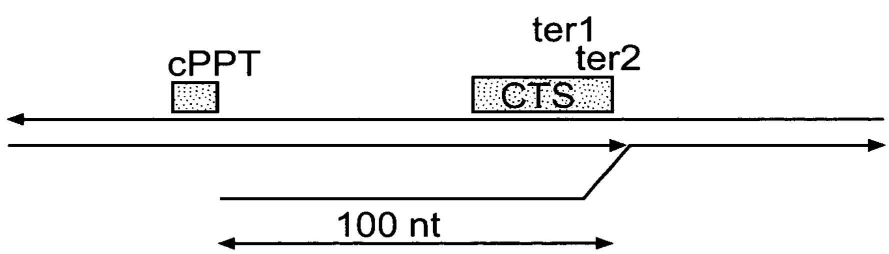
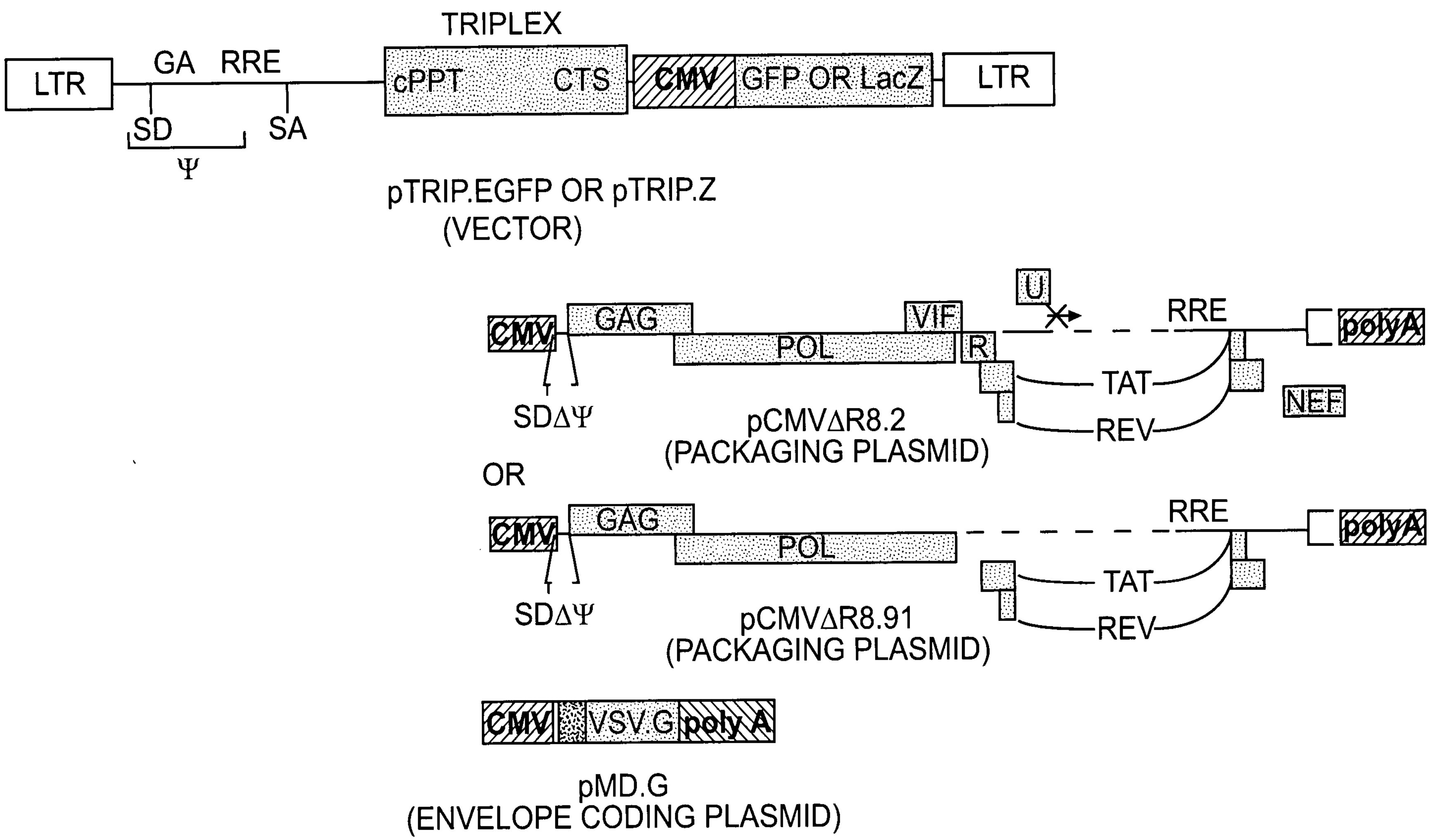


FIG. 1A



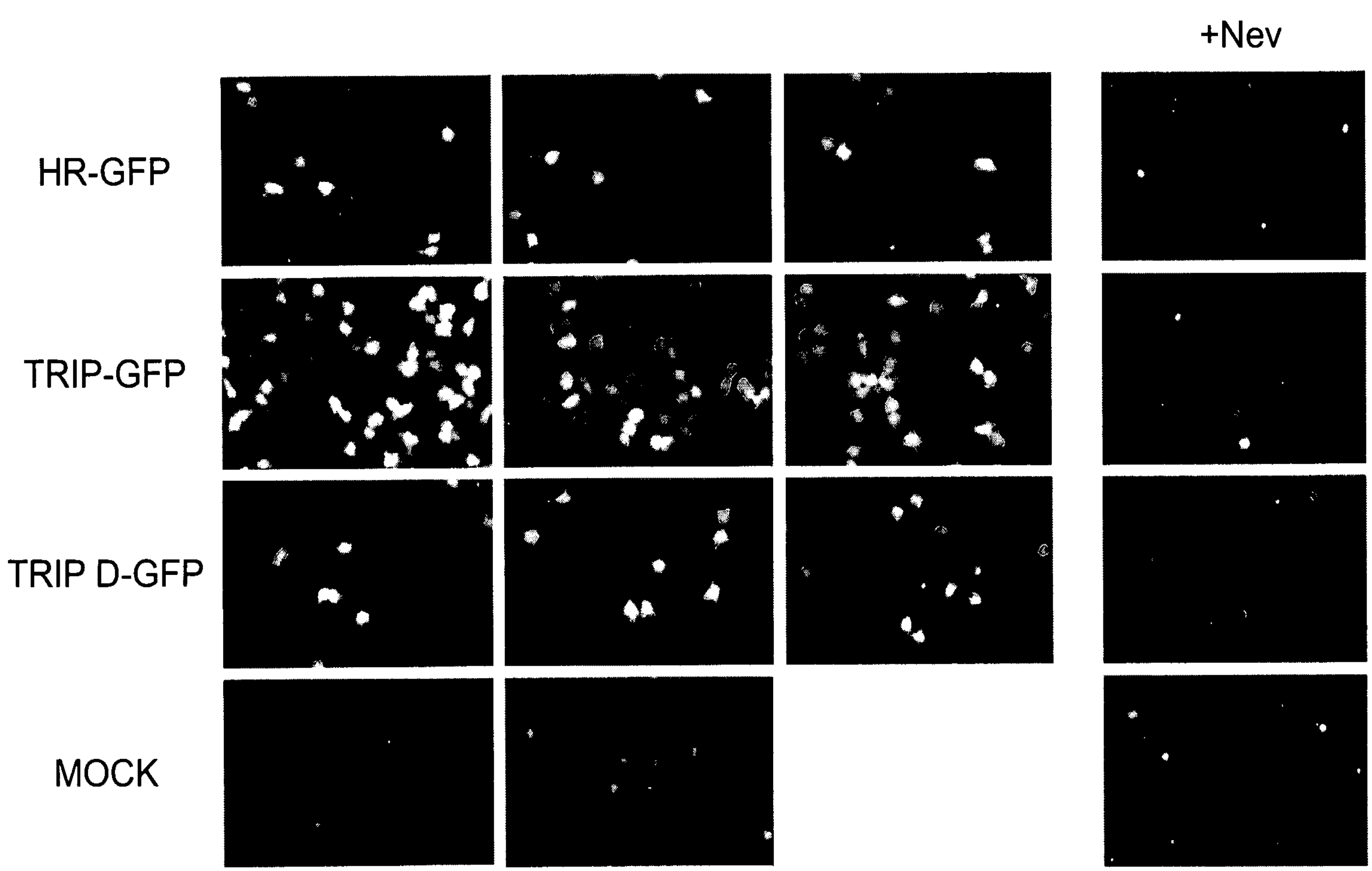
REVERSE TRANSCRIPTION OF LENTIVIRUSES,
FORMATION OF CENTRAL DNA TRIPLEX

FIG. 1B



PLASMIDS USED FOR THE PRODUCTION OF HIV VECTOR PARTICLES

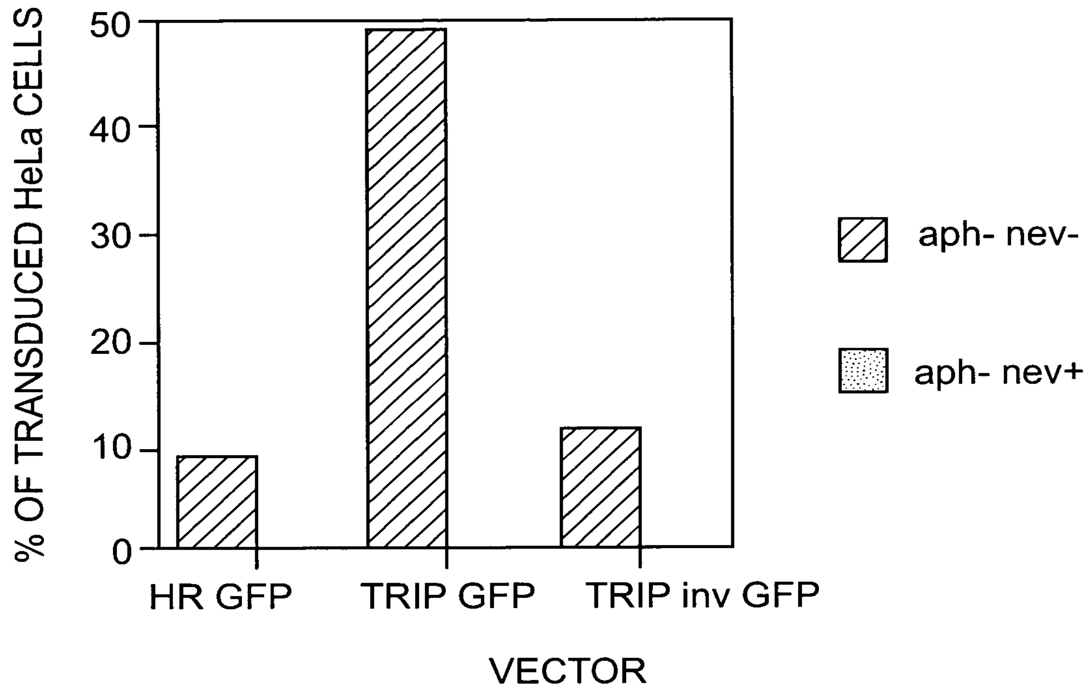
FIG. 2



IMPACT OF TRIPLEX ON EGFP TRANSDUCTION IN HeLa CELLS

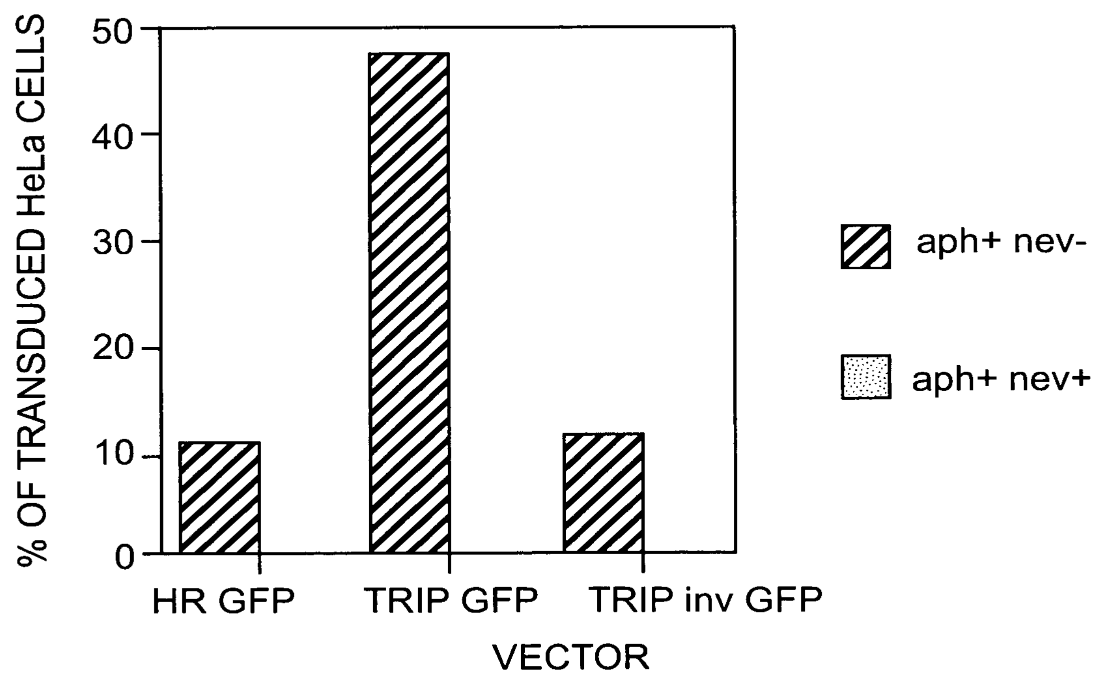
FIG. 3

QUANTIFICATION OF DEGREE OF TRANSDUCTION OF EGFP GENE
BY HIV VECTORS WITH OR WITHOUT TRIPLEX



TRANSDUCTION OF GFP IN MITOTIC HeLa CELLS

FIG. 4A



TRANSDUCTION OF GFP IN BLOCKED HeLa CELLS

FIG. 4B

IMPACT OF TRIPLEX ON TRANSDUCTION OF DIVIDING OR NONDIVIDING Hel CELLS, WITH GFP

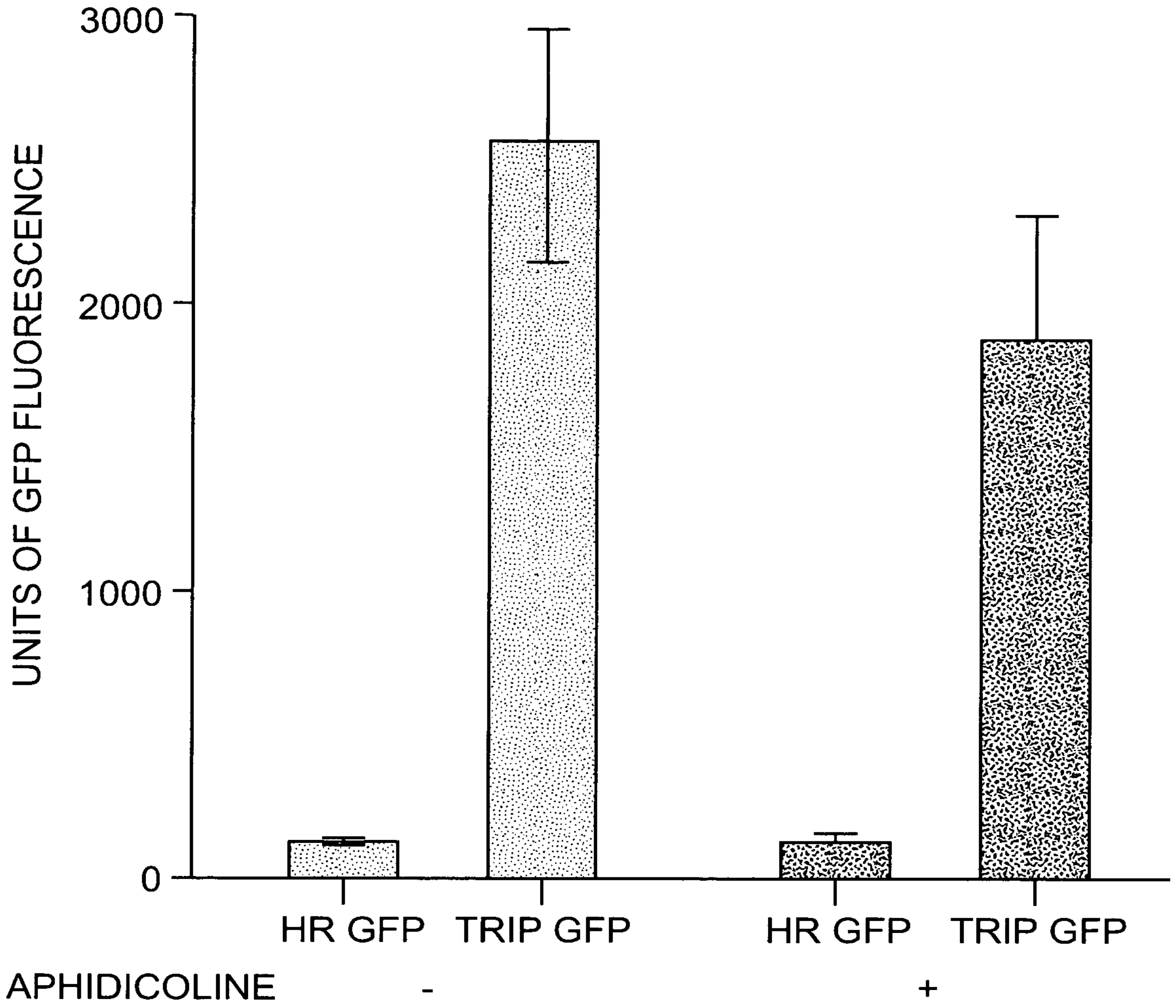
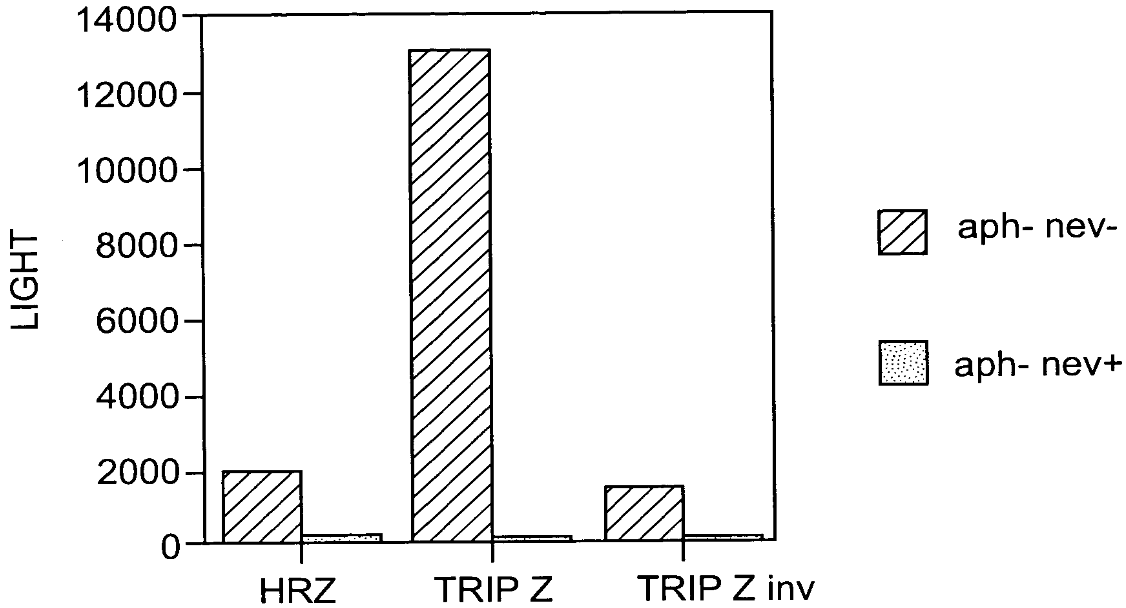


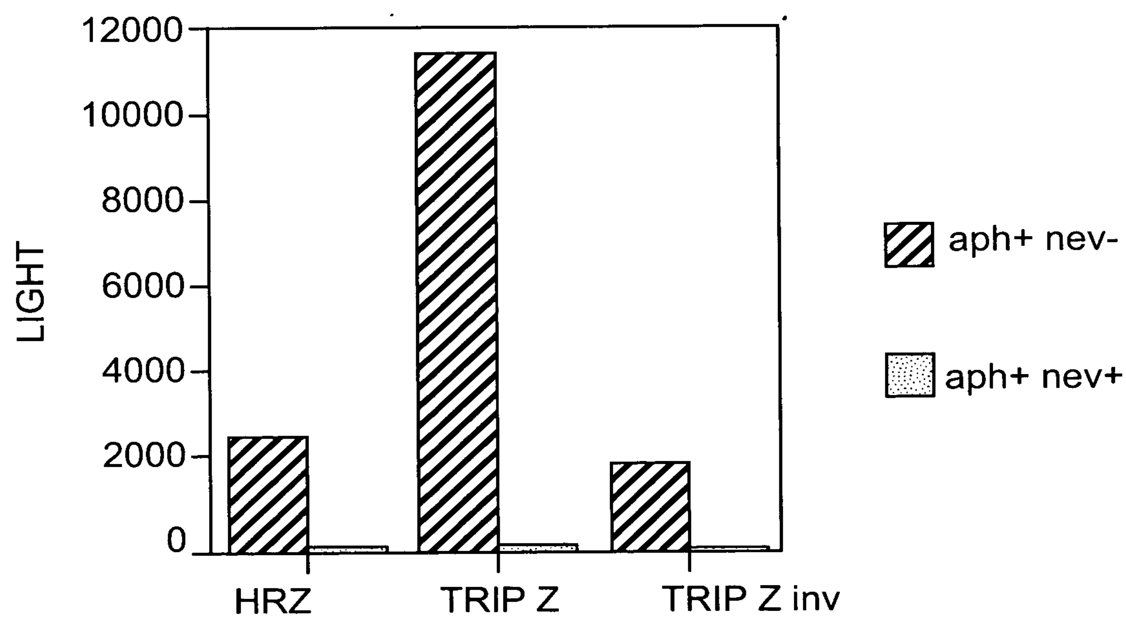
FIG. 4C

QUANTIFICATION OF DEGREE OF TRANSDUCTION OF LacZ GENE
BY HIV VECTORS WITH OR WITHOUT TRIPLEX



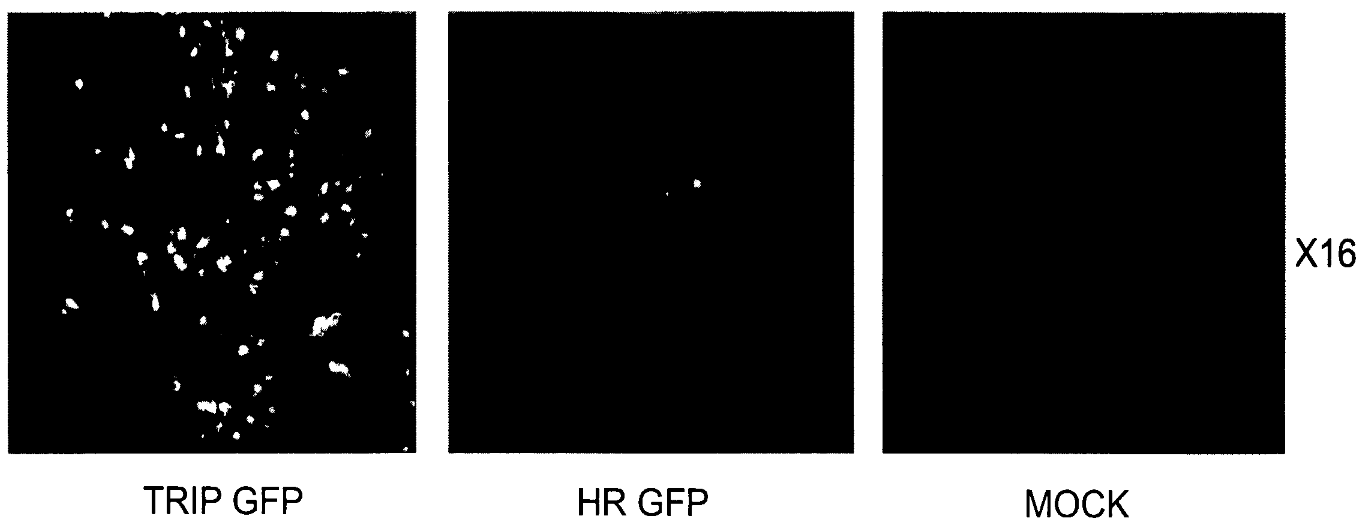
TRANSDUCTION OF βGAL IN MITOTIC HeLa CELLS

FIG. 5A



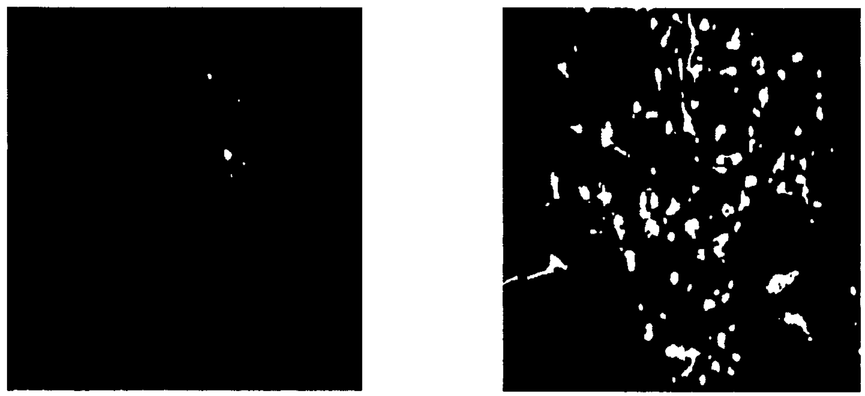
TRANSDUCTION OF βGAL IN NON MITOTIC HeLa CELLS

FIG. 5B



IMPACT OF CENTRAL TRIPLEX ON TRANSDUCTION OF GFP GENE IN RAT PRIMARY SPINAL CELLS

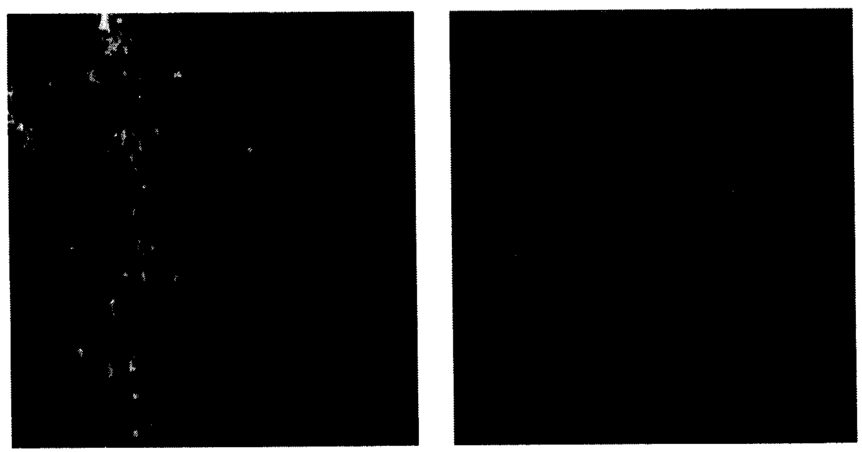
FIG. 6A



HR GFP TRIP GFP

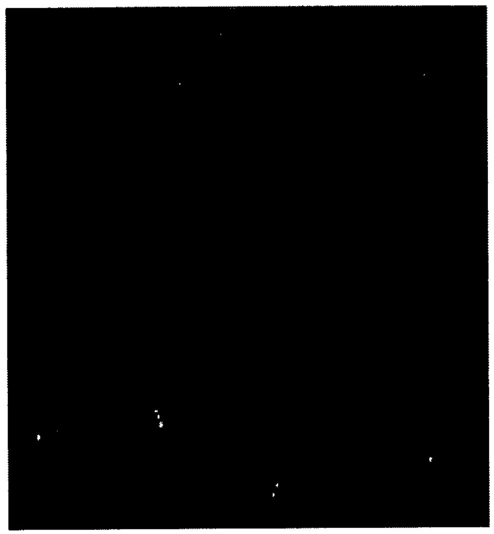
IMPACT OF CENTRAL TRIPLEX ON TRANSDUCTION OF GFP GENE IN RAT PRIMARY SPINAL CELLS

FIG. 6B



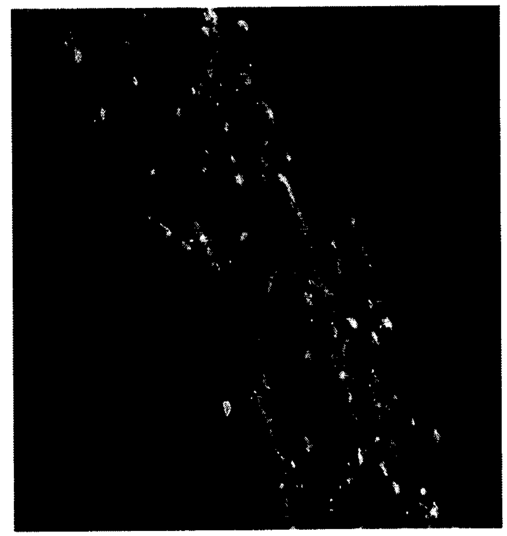
IMPACT OF TRIPLEX ON IN VIVO TRANSDUCTION OF
EGFP GENE IN RAT BRAIN: TRANSDUCTION AT INJECTION SITE

FIG. 7A.1



HR GFP

A



TRIP GFP

B

IMPACT OF TRIPLEX ON IN VIVO TRANSDUCTION OF GFP GENE IN RAT BRAIN

FIG. 7A.2

IMPACT OF TRIPLEX ON TRANSDUCTION OF LUCIFERASE ACTIVITY IN HeLa CELLS IN VITRO

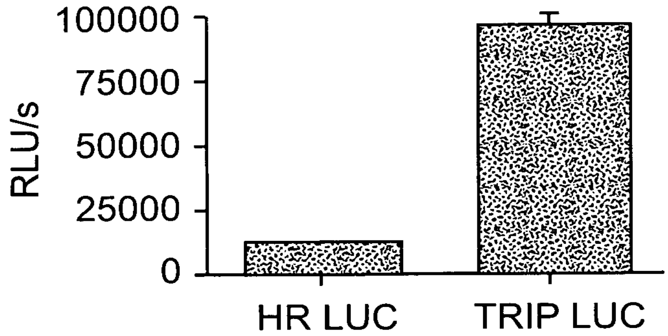


FIG. 7B.1

IMPACT OF TRIPLEX ON TRANSDUCTION OF LUCIFERASE ACTIVITY IN RAT BRAIN IN VIVO

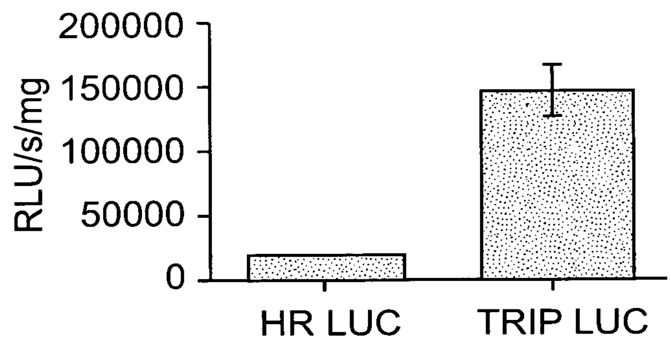


FIG. 7B.2

IMPACT OF TRIPLEX ON TRANSDUCTION OF LUCIFERASE ACTIVITY IN MOUSE BRAIN CELLS IN VIVO

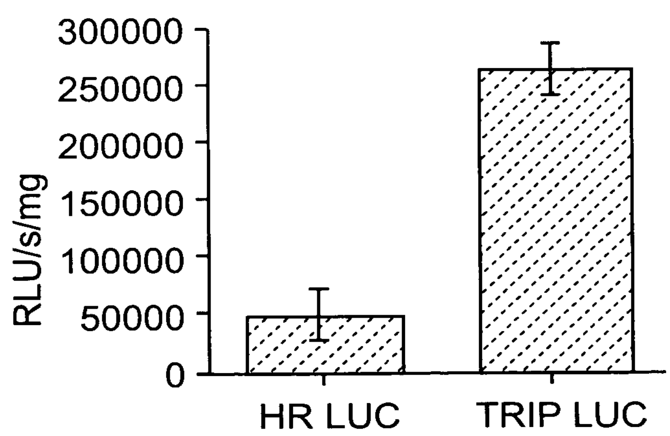


FIG. 7B.3

METHOD FOR QUANTITATIVE ANALYSIS OF MATURATION OF VECTOR DNA

A) SOUTHERN BLOT STRATEGY

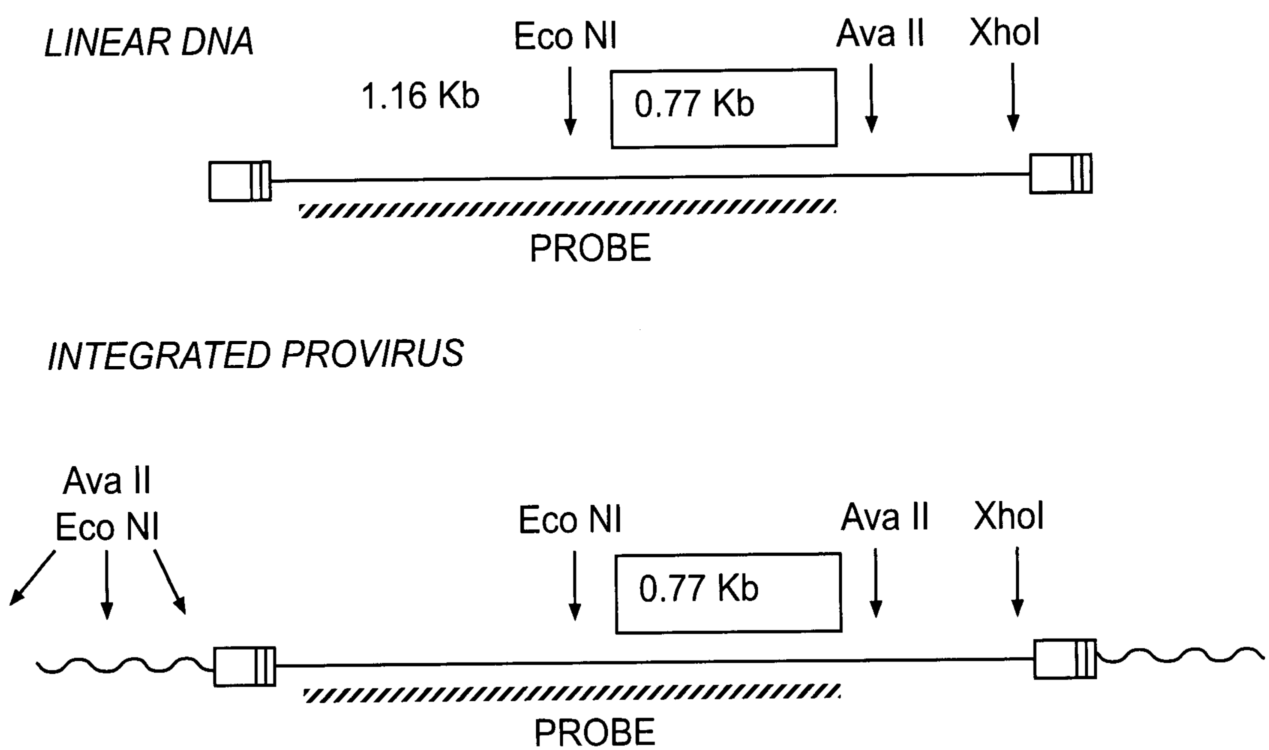
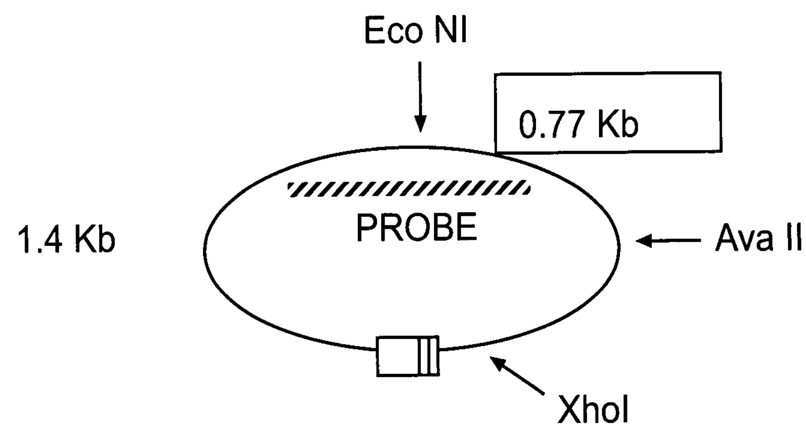
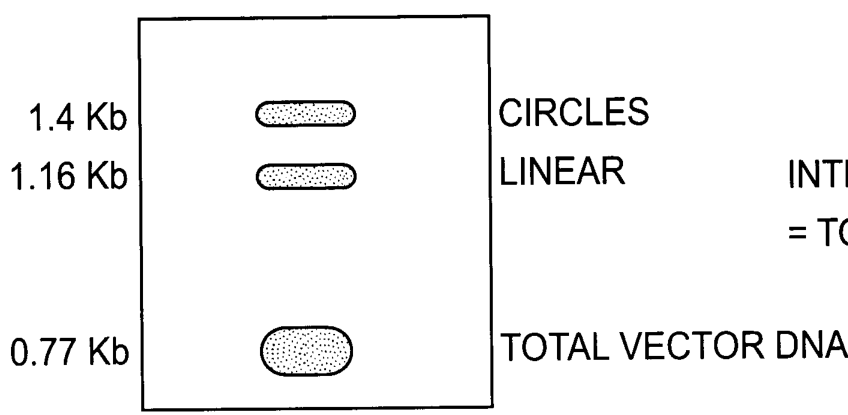


FIG. 8A

NON INTEGRATED DNA CIRCLES



B) QUANTIFICATION BY PHOSPHORIMAGE



INTEGRATED PROVIRUS
= TOTAL VECTOR DNA-LINEAR-CIRCLES

FIG. 8B

ANALYSIS OF NUCLEAR IMPORT OF VECTOR DNA

FIG. 9A

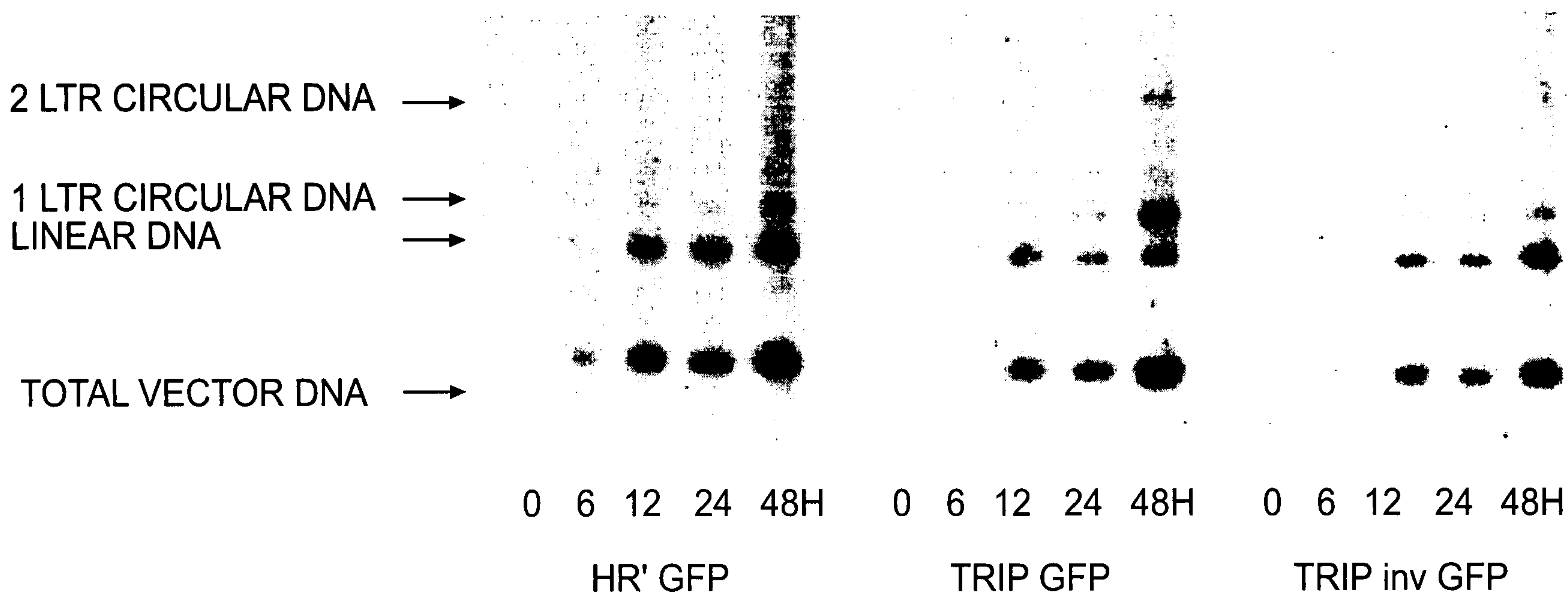


FIG. 9B

BEST AVAILABLE COPY

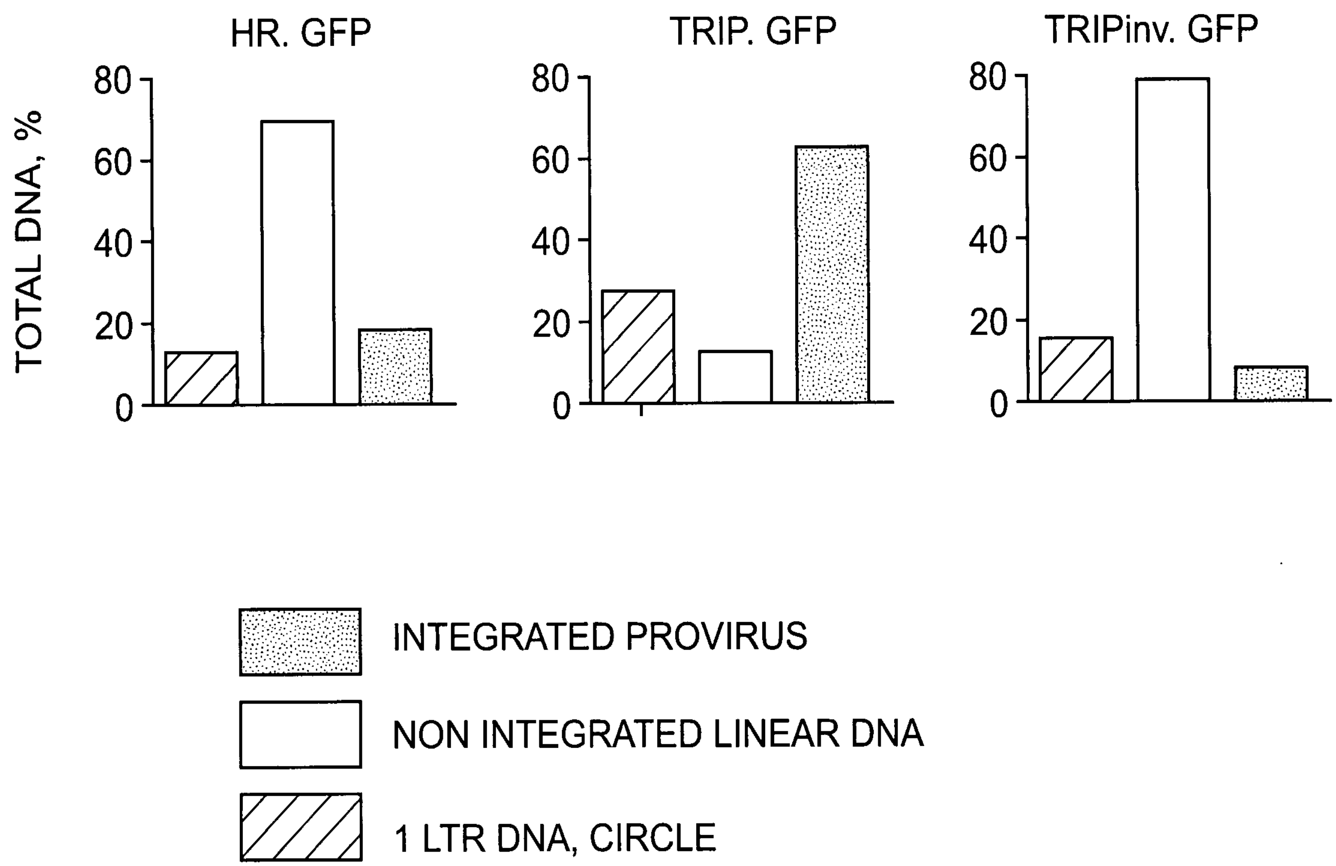
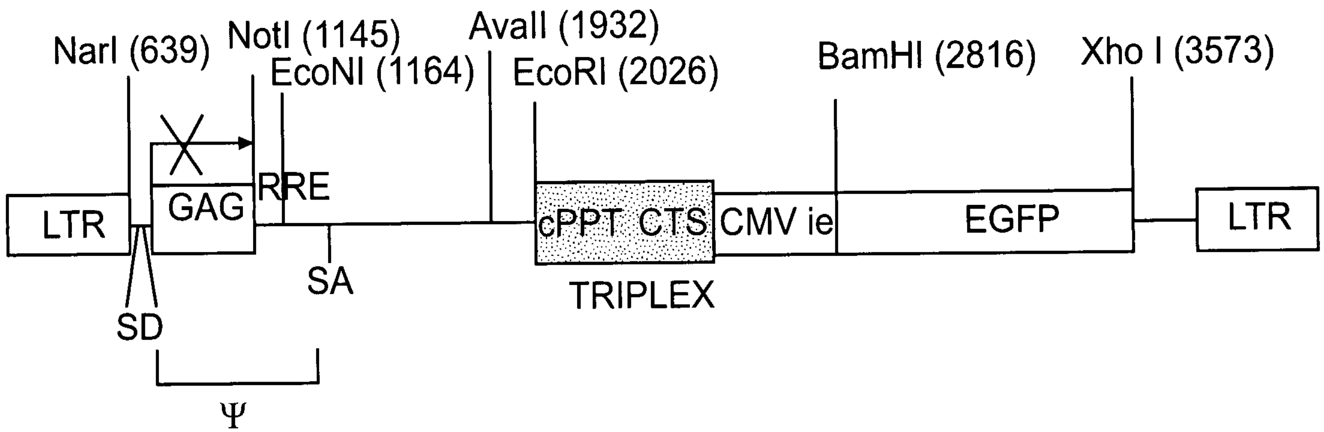

HR. GFP
TRIP. GFP
TRIPinv. GFP
TOTAL DNA, %
80
60
40
20
0
INTEGRATED PROVIRUS
NON INTEGRATED LINEAR DNA
1 LTR DNA, CIRCLE

FIG. 9C



HIV A TRIPLEX VECTOR: TRIP-EGFP

FIG. 10

TRIPLEX CAEV (CAPRINE ARTHRITIS ENCAPHALITIS VIRUS)

```
                                   BspW I
                                Fnu4H I
              Tfi I          Fnu4H I
              Hinf I         Bbv I      Rma I
           Sfe I     BspW I    Bbv I
gttccagccacaatttgtcgctgtagaatcagccatagcagcagccctagtcgccataaatataaaaagaaagggtggctggggacaagccctatggat  100
caaggtcggtgttaaacagcgacatcttagtcggtatcgtcgggatcagcggtatttatatttttctttcccacccgacccctgttcgggataccta
           21       32     41     47
             26       38
             26       38
                        41
                          44
                                                 cPPT

                                                                                        Mnl I
attttatatatataataaagaacagaaaagaataaataataaatataataaaaattctcaaaaaattcaattctgttattacagaataaggaaaagaggac  200
taaaaatatatattatttcttgtcttttcttatttattatttatattatttttaagagtttttaagttaagacaataatgtcttattccttttctcctg
                                                                                        195
                                            <<CTS>>
```

FIG. 11A

TRIPLEX EIAV (EQUINE INFECTIOUS ANAEMIA VIRUS)

```
                                   Nla III
                                   Sty I
                                   Nco I
                                   Dsa I                                                  Tth111 II
Mae III   Mnl I       Mnl I        BsaJ I          Tth111 II     Nla III      Sfe I
CTTGTAACAAAGGGAGGGAAAGTATGGGAGGACAGACACCATGGGAAGTATTTATCACTAATCAAGCACAAGTAATACATGAGAAACTTTTACTACAGCA 100
GAACATTGTTTCCCTCCCTTTCATACCCTCCTGTCTGTGGTACCCTTCATAAATAGTGATTAGTTCGTGTTCATTATGTACTCTTTGAAAATGATGTCGT
  4       14          28           39              63            78           93   99
         cPPT                      39
                                   39
                                   39
                                    40

                                     Hph I
                                   ScrF I
                                   EcoR II
                                   Dsa V                   Sau96 I
                                   BstN I                  Ava II
                                   BstK I                 PpuM I
   Mnl I                           BsaJ I    Nla III      Nla IV   Bma I           Hph I   Bsg I
AGCACAATCCTCCAAAAAATTTTGTTTTTACAAAATCCCTGGTGAACATGATTGGAAGGGACCTACTAGGGTGCTGTGGAAGGGTGATGGTGCAGTAGTA 200
TCGTGTTAGGAGGTTTTTTAAAACAAAAATGTTTTAGGGACCACTTGTACTAACCTTCCCTGGATGATCCCACGACACCTTCCCACTACCACGTCATCAT
   109                             137       147          158      166             183     190
            <<CTS>>                 138                   158
                                    138                   158
                                    138                    159
                                    138                    159
                                    138
                                      141
```

FIG. 11B

TRIPLEX VISNA

```
     Mnl I
 Sau96 I
 Nla IV                                Rma I                                    Mse I
 Ava II
 |   |                                 |                                        |
 GGACCCTCATTACTCTAAATATaaaaagaaagggTGGGCTAGGGACAAGCCCTATGGATATATTTATATTTAATAAGGAACAACAAAGAATACAGCAACA 100
 CCTGGGAGTAATGAGATTTATAttttctttcccACCCGATCCCTGTTCGGGATACCTATATAAATATAAATTATTCCTTGTTGTTTCTTATGTCGTTGT
 |   |    .        .        .       |         .        .        |         .        .        .
 1                                    39                          70
 1
 1                   cPPT
    5

                                                                              ScrF I
                                                                              EcoR II
                                                                              Dsa V
                                                                              BstX I
                                                                              BstN I
                                                                           Fok I
                                                                          SfaN I               Sau96 I       Rsa I
              Xmn I     Taq I                                       Mnl   I   BstK I           Ava II        Csp6 I
              |         |                                           |     ||  |                |             |
AAGTAAATCaaaacaagaaaaaattcGATTTTGTTATTACAGAACAAGAAAAAGAGGGCATCCAGGAGAGTGGCAAGGACCAACACAGGTACTTTGGGGC 200
TTCATTTAGttttgttcttttttaagCTAAAACAATAATGTCTTGTTCTTTTTCTCCCGTAGGTCCTCTCACCGTTCCTGGTTGTGTCCATGAAACCCCG
         .    |   .     |   .         .         .         |     ||. |       .       |   .          |          .
              117       125                                 154       162             177            189
                                                                  158                 177            189
       <<CTS>>                                                     159
                                                                          162
                                                                          162
                                                                          162
                                                                          162
                                                                          162
```

FIG. 11C

TRIPLEX SIV_{AGM} (SIMIAN IMMUNODEFICIENCY VIRUS)

```
                             Mse I                                           Ase I
                             Dra I      Mnl I               Eco57 I BsmA I    Mse I
TACTGATGGCTTGCATACTTCACAATTTTAAAAGAAAGGGAGGAATAGGGGGACAGACTTCAGCAGAGAGACTAATTAATATAATAACAACACAATTAGA 100
ATGACTACCGAACGTATGAAGTGTTAAAATTTTCTTTCCCTCCTTATCCCCCTGTCTGAAGTCGTCTCTCTGATTAATTATATTATTGTTGTGTTAATCT
                             27         40                  58      68       76
                              28                                             75
                                    cPPT
```

```
                                                                                     Msp I
                                                                                     Hpa II
                                                  Acc I                             Cfr10 I
                                   Mse I       Ple I  Sfe I          BsmA I       Sau96 I      Mse I
                                  Dra I        Hinf I                Bsa I        Ava II       Ase I
AATACAACATTTACAAACCAAAATTCAAAAAATTTTAAATTTTAGAGTCTACTACAGAGAAGGGAGAGACCCTGTGTGGAAAGGACCGGCACAATTAATC 200
TTATGTTGTAAATGTTTGGTTTTAAGTTTTTTAAAATTTAAAATCTCAGATGATGTCTCTTCCCTCTCTGGGACACACCTTTCCTGGCCGTGTTAATTAG
                                  134          145                   166          183          194
                                   135         145    152            166          183           195
                               <<CTS>>          147                                 185
                                                                                     186
                                                                                     186
```

FIG. 11D

TRIPLEX HIV-2 RID (HUMAN IMMUNODEFICIENCY VIRUS)

```
                                                                                Hph I
                                                                               Sau3A I
                                                                               Mbo I
       Mse I                                                                   Dpn II
       Dra I                          Ple I               Mse I                Dpn I
 Nla III                              Hinf I             Ase I               Bcl I                           Mnl I
 |      ||                            |                  ||                  || |                           |
tgcatgaatttaaaagaaggggggggaataggggatatgactccatcagaaagattaatcaatatgatcaccacagaacaagagatacaattcctccaag 100
acgtacttaaattttcttccccccccttatcccctatactgaggtagtctttctaattagttatactagtggtgtcttgttctctatgttaaggaggttc
 |      ||       .         .          |.         .     ||     .     || | .         .         .|         .
 3                                    39                 54          65                             93
       10                             39                  55          66
        11                                                            66
                cPPT                                                  66
                                                                      66
                                                                       68
```

FIG. 11E

TRIPLEX HIV-2 RID (HUMAN IMMUNODEFICIENCY VIRUS)

```
                                                                         ScrF I
                                                                         EcoR II
                                                                         Dsa V
                                                                         BstN I
                                                                         BstK I
                                                                         BsaJ I
                                                                      Sau96 I
                                                                      Ava II
                                                                     PpuM I
                                                  Sau3A I            EcoO109 I
              Mse I                               Mbo I              || |
              |                                   Dpn II             || |
              |                                   Dpn I              || |
ccaaaaattcaaaattaaaagattttcgggtctatttcagagaaggcagagatcagttgtggaaaggacctggggaactactgtggaaaggagaaggagc 200
ggtttttaagttttaattttctaaaagcccagataaagtctcttccgtctctagtcaacacctttcctggaccccttgatgacacctttcctcttcctcg
    .    |    .         .         .        .|       .       ||  |.        .         .         .
         115                                151                165
                                            151                165
      <<CTS>>                               151                 166
                                            151                 166
                                                                   169
                                                                   169
                                                                   169
                                                                   169
                                                                   169
                                                                   169
```

FIG. 11E (cont)

TRIPLEX HIV-1 LAI

```
                                                   Bsg I
                Mse I                      Rsa I
 Fok I         Dra I                       Csp6 I              Acc I
   |           ||                          |   |               |
CAGTATTCATCCACAAtttaaaagaaaaggggggATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAAACTAAAGA 100
GTCATAAGTAGGTGTTaaaattttcttttccccccTAACCCCCCATGTCACGTCCCCTTTCTTATCATCTGTATTATCGTTGTCTGTATGTTTGATTTCT
   |           ||                          |   |               |
   8           18                          44                  66
                19                         44
                      cPPT                     49
```

```
                                                         Sau3A I
                                                         Mbo I
                                                         Dpn II
                                                         Dpn I
                                                         Alw I
                                                        BstY I       Sau96 I         Mnl I
                                                                     Ava II      Alu I
                                                        ||           |           |   |
ATTACAAAAACAAATTACaaaaattcaaaattttCGGGTTTATTACAGGGACAGCAGAGATCCACTTTGGAAAGGACCAGCAAAGCTCCTCTGGAAAGGT 200
TAATGTTTTTGTTTAATGtttttaagttttaaaaGCCCAAATAATGTCCCTGTCGTCTCTAGGTGAAACCTTTCCTGGTCGTTTCGAGGAGACCTTTCCA
                                                        ||           |           |   |
                                                        158          174         184
                                                         159         174             188
         <<CTS>>                                         159
                                                         159
                                                         159
                                                         159
```

FIG. 11F

5′ TTTTAAAAGAAAAGGGGGGATTG-

cPPT

-GGGGGTACAGTGCAGGGGAAAGAATAG-

-TAGACATAATAGCAACAGACATACAAA-

-CTAAAGAATTACAAAAACAAATTAC-

-AAAAATTCAAAATTTTC 3′

CTS

TRIPLEX DNA REGION OF HIV-1 VIRUS

FIG. 11G

ALIGNMENT OF cPPT AND 3′PPT SEQUENCES
IN SOME LENTIVIRUSES

3′ PPT	AAAAGAAAAGGGGGG	*HIV-1*
CENTRAL PPT	***************	
	AAAACAAGGGGGGG	*HIV-2 ROD*
	****G*********	
	AAAAGAAAAGGGGGG	*SIV mac & HIV-2 NIH-Z*
	*******GG**A**A	
	AAAAGAAAAGGGAGG	*SIVagm*
	********G**AG*A	
	AAAAAGAAAAAAGAAAGGGTGG	*VISNA*
	T*T*************	
	AAAAATAAAAAAAGAAAGGGTG	*CAEV*
	T***************	
	AACAAGGGGGAA	*EIAV*
	AGG*A*A**	

FIG. 11H

INDUCTION OF PRIMARY CTL RESPONSES USING HUMAN DENDRITIC CELLS TRANSDUCED BY THE TRIP-MEL-IRES-GFP VECTOR

PRIMARY CTL INDUCTION USING DENDRITIC CELLS TRANSDUCED BY MSL LENTIVIRUS

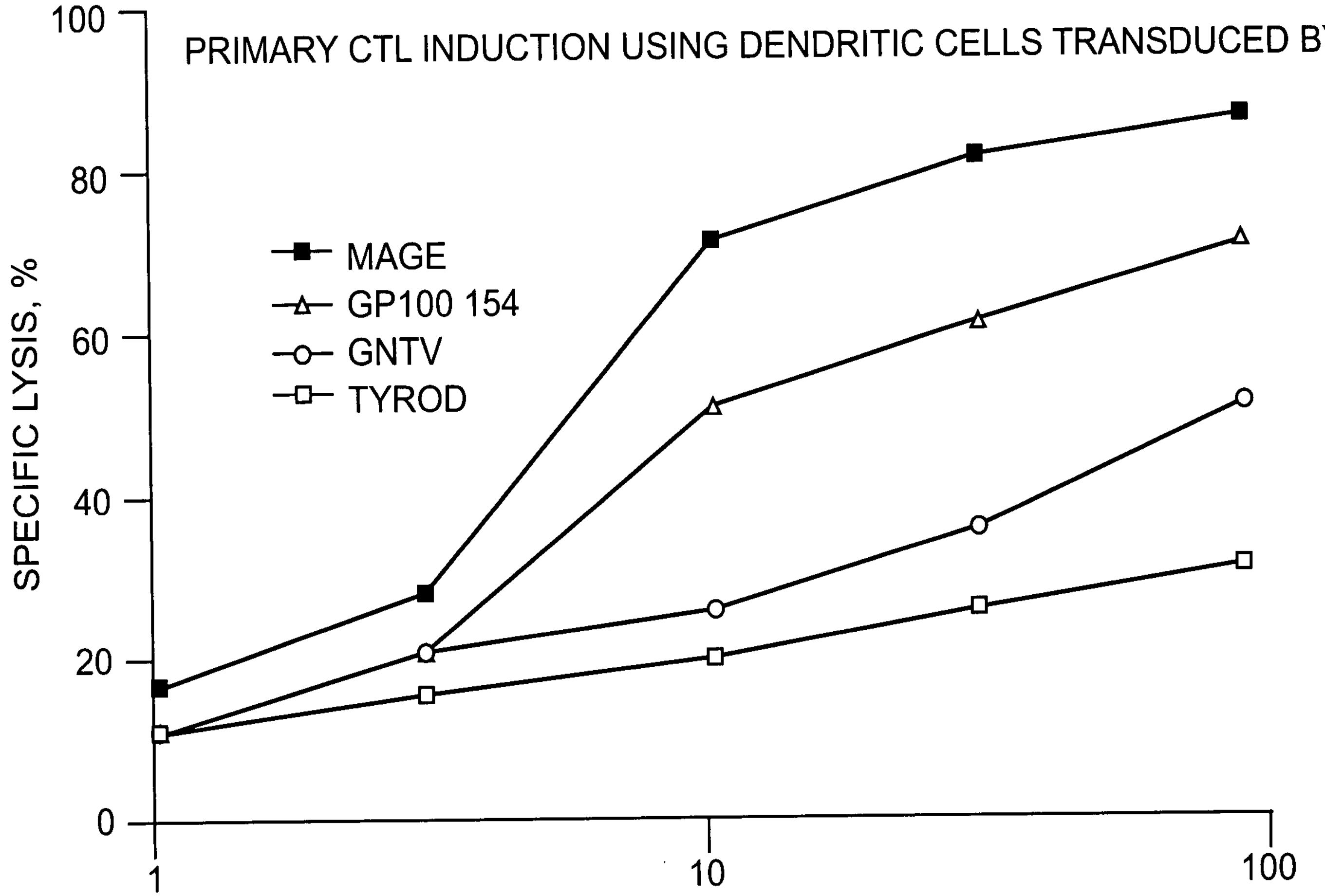
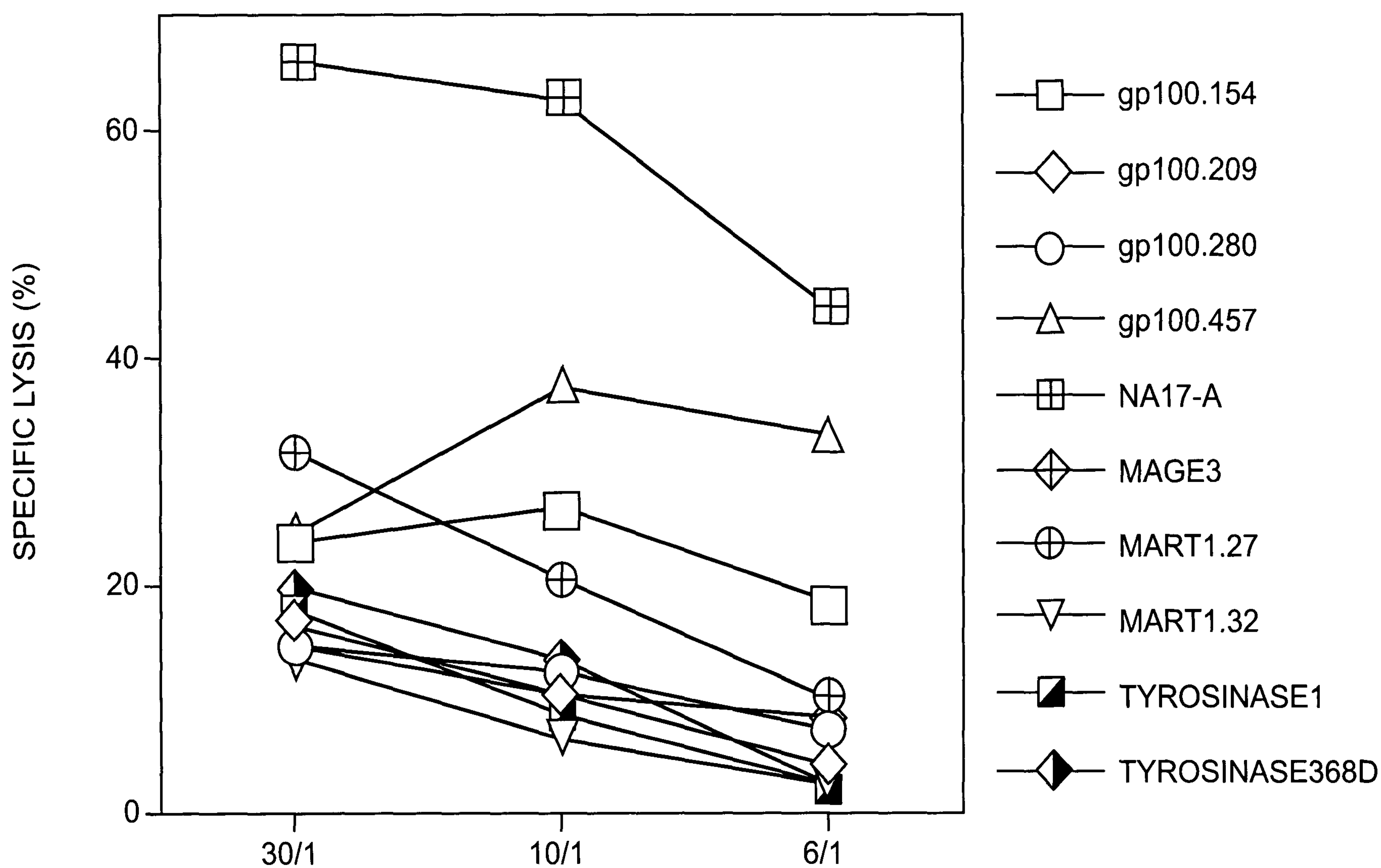


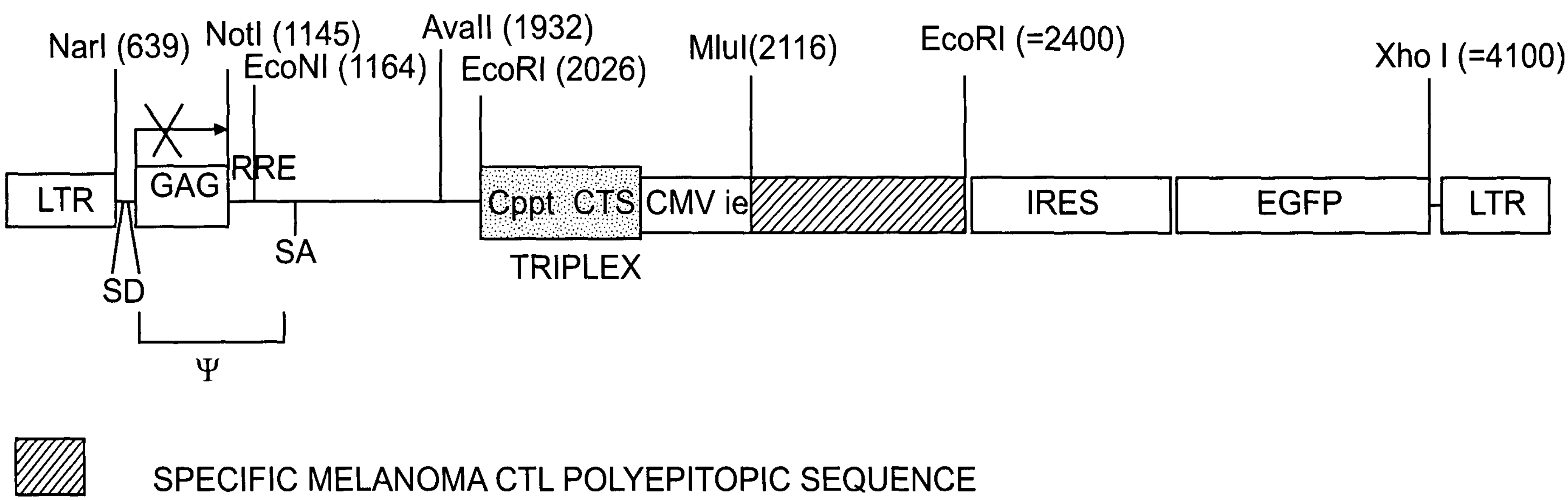
FIG. 12



RESULTS REPRESENTING THE CYTOTOXIC RESPONSE AFTER IMMUNIZATION (INTRAPERITONEAL) OF HHD MICE BY THE TRIP-MEL-IRES-GFP VECTOR

FIG. 13

RESTRICTION MAP OF pTRIP.MEL-IRES-GFP



SOURCE: CHARNEAU
HOST: JM109

FIG. 14

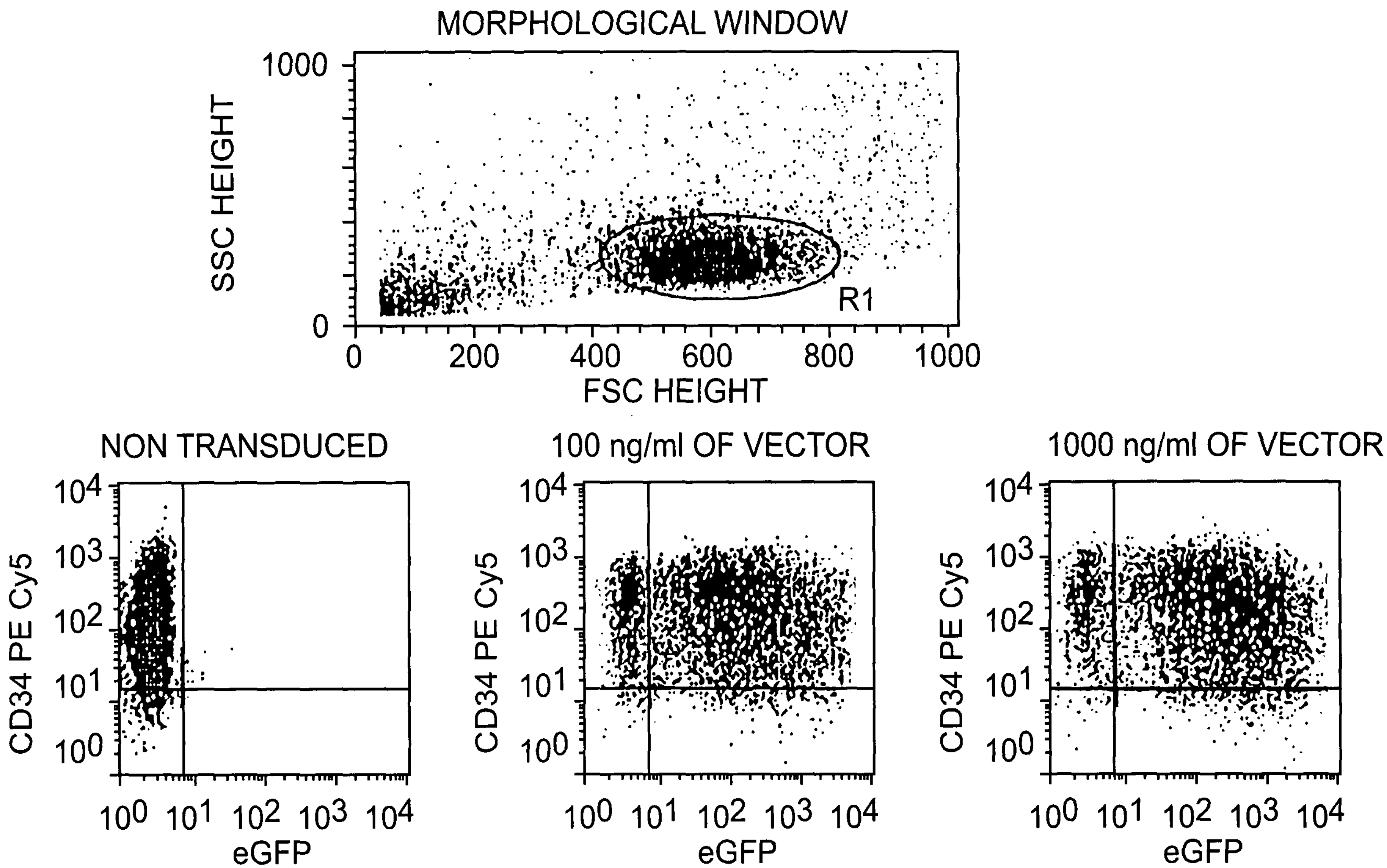
EPITOPIC PEPTIDES INCLUDED IN MELANOMA POLYEPITOPE

MELANOMA PEPTIDE		SEQUENCE	REFERENCE	
gp100	154-162	KTWGQYWQV	KAWAKAMI, Y. ET AL.	J.IMMUNOL.1995.154:3961-8.
	209-217	ITDQVPFSV	KAWAKAMI, Y. ET AL.	J.IMMUNOL.1995.154:3961-8.
	280-288	YLEPGPVTA	COX, AL. ET AL.	SCIENCE.1994.264:716-9.
	457-466	LLDGTATLRL	KAWAKAMI, Y. ET AL.	J.IMMUNOL.1995.154:3961-8.
MART-1	27-35	AAGIGILTV	KAWAKAMI, Y. ET AL.	J.IMMUNOL.1995.154:3961-8.
	32-40	ILTVILGVL	CASTELLI, C. ET AL.	J.EXP.MED.1995.181:363-8.
TYROSINASE	1-9	MLLAVLYCL	WOLFEL, T. ET AL.	EUR.J.IMMUNOL.1994.24:759-64.
	368-376-D	YMDGTMSQV	MOSSE, CA. ET AL.	J.EXP.MED.1998.187:37-48.
GnT-V/NA17-A	nt38-64b	VLPDVFIRC	GUILLOUX, Y. ET AL.	J.EXP.MED.1996.183:1173-83.
MAGE-3	271-279	FLWGPRALV	VAN DER BRUGGEN, P. ET AL.	EUR.J.IMMUNOL.1994.24:3038-43.

AMINO ACID SEQUENCE OF MELANOMA POLYPITOPE

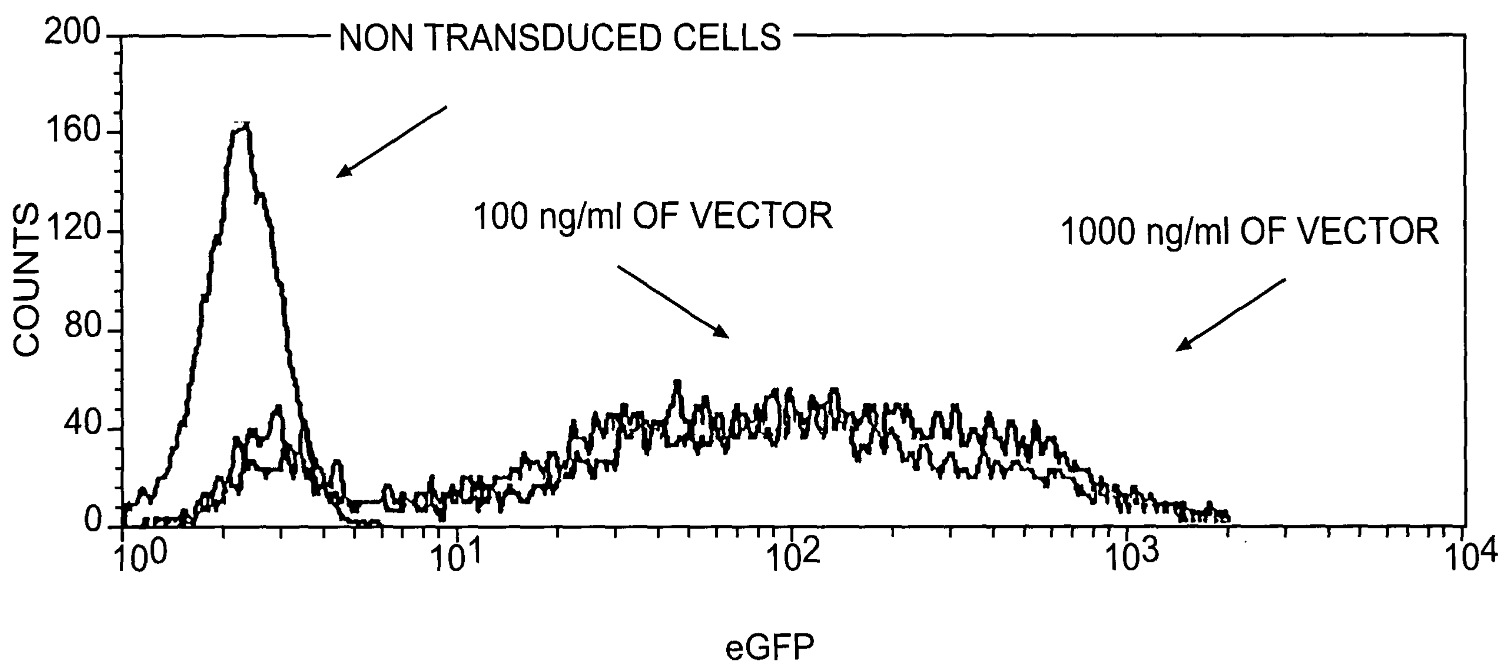
AAGIGILTV<u>FLWGPRALV</u>MLLAVLYCL<u>LLDGTATLRL</u>KTWGQYWQV<u>YMDGTMSQV</u>ITDQVPFSV<u>YLEPGPVTA</u>ILTVILGVL<u>VLPDVFIRCV</u>

FIG. 15



VERY HIGH EFFICIENCY TRANSDUCTION OF CD34+ STEM CELLS
BY TRIPLEX HIV A VECTORS
ANALYSIS ON POST TRANSDUCTION DAY 5

FIG. 16A



VERY HIGH EFFICIENCY TRANSDUCTION OF CD34+ STEM CELLS
BY TRIPLEX HIV A VECTORS
ANALYSIS ON POST TRANSDUCTION DAY 5

FIG. 16B